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THE MOTILE BEHAVIOR OF VIRUS-TRANSFORMED 3T3 CELLS

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Abstract

Using metallic gold in various assays for the motility of cultured tissue cells, the paper compares the movements of surface projections and the locomotion of polyoma (Py3T3) and SV40 (SV3T3) virus-transformed 3T3 cells with the behavior of the parental 3T3 cells.

The movement of surface projections was assayed by the ability of filopodia, lamellipodia and blebs of freshly plated cells to remove colloidal gold particles from a particle-coated glass substrate. The ability of filopodia to probe the environment for points of anchorage was tested by observing cells plated on glass whose filopodia touched the surface of a neighboring area of evaporated gold. The locomotion of cells was assayed by particle-free tracks (phagokinetic tracks) which were left by migrating cells on a glass substrate which was previously coated with colloidal gold particles.

The paper suggests that the ability of the transformed cells to sense environmental factors, and their behavioral controls are altered.

KEY WORDS: Locomotion, surface projections, fibroblasts, gold, phagokinetic tracks.

Introduction

It is generally believed that some malignant tumors such as mammary carcinomas require many months or even years to proceed from the single cell stage to clinically detectable metastases. Furthermore, about 90% of human neoplasias are carcinomas (Straeuli, 1985) which originate from epithelial cells which migrate very slowly as individual cells (DiPasquale, 1975, Albrecht-Buehler, 1979). Therefore, the frequently expressed assumption that malignant cells are more motile than normal ones cannot be generally assumed to be true. On the contrary, many highly invasive cell types appeared to be almost immobilized within the tissue of mesentery in organ culture (Haemmerli, 1985). Dissemination of metastatic cells seems to involve little active locomotion of individual cells, but rather induction of angiogenesis (Folkman and Haudenschild, 1980), invasion of blood vessels (Nicosia et. al., 1983)), and passive transport via blood or lymph to the secondary sites of their growth (for review see Nicholson, 1982). Locomotion of individual malignant cells seems to be required only for relatively short distances as the cells cross basement membranes in vivo or in vitro (Starkey et. al., 1984), the walls of venules or of lymphatic vessels. Further invasion into solid tissue seems to be achieved by the growth of the metastasis.

If the motile activity of malignant cells is not generally increased, invasive behavior may be explained by the assumption of altered sensitivities to environmental clues and to signals from surrounding tissues. As a result, one can expect that the behavior of malignant cells is altered. Unfortunately, the behavior of malignant cells cannot be observed in vivo with our present microscopic methods. Therefore, a number of in vitro studies with transformed

cultured cells or tumor explants have been carried out instead. They included the observation of invasion of cell populations growing in culture (Abercrombie and Heaysman, 1976), the induction of in vitro angiogenesis by tumor-conditioned medium in cultured capillary endothelial cells (Folkman and Haudenschild, 1980), the study of the alterations of the ability of transformed cells to make contacts with the substrate and to develop them into mature focal contacts (for review see Vasiliev, 1985), the study of alterations of the cytoarchitecture of transformed cells (for review see Ben-Ze'ev, 1985), and many other expressions of cancer cells, that reflect in vitro their altered behavior and ability to respond normally to their tissue environment in vivo.

One may even go further and interpret the motile behavior of cultured cells as an expression of 'cytoplasmic intelligence', i.e. the ability of cells (a) to collect and integrate a large number of simultaneously received data, (b) to make decisions and (c) to coordinate many complex functions in a purposeful way (Albrecht-Buehler 1985). In view of this concept, one may speculate that the invasive behavior of malignant cells is a fundamental alteration in the 'intelligent' control systems of cell motility. In order to test whether this hypothesis is reasonable, the present paper examined various motile responses of two virus-transformed 3T3 cells to their environment and compared them with their 'normal' counterpart. A number of differences were observed. The paper suggests that they may reflect alterations of the ability of the cells to assess their environment and to respond to it.

Materials and Methods

Cell cultures

Swiss 3T3, Py3T3 and SV3T3 cells were grown in Dulbecco's Modified Eagle's Medium (DME) supplemented with 10% calf serum (CS) at 37°C in a 10% CO_2 atmosphere with saturated humidity. The cells were grown on Falcon plastic dishes (Becton-Dickinson, Oxnard, CA) and sub-cultured every 3 days.

Preparation of colloidal gold particles

The preparation of gold particles and the coating of glass coverslips with particles has been described in detail elsewhere (Albrecht-Buehler 1977b).

Assay for pseudopodia motility

The assay has been described earlier. (Albrecht-Buehler and Lancaster 1976). Briefly, cells grown for two days in DME + 10% CS were removed by 0.5 mM EDTA + 0.05% trypsin in phosphate buffered saline (PBS) and resuspended in DME + 10% CS. They were spun for 3 min at 1,000 rpm in a table top centrifuge and resuspended three times in washing solution (140 mM NaCl and 3mM KCl in water). Approximately $3x10^4$ cells were plated into prewarmed 3.5 cm Falcon plastic dishes containing normal salt solution (113 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 15 mM Na-phosphate (pH= 7.4) and 15 ug/ml phenol red in quartz distilled water) and 2 circular glass cover slips coated with gold particles. The dishes were transferred to incubators and the CO_2-flow turned off in order to prevent acidification of the buffered test solutions. After 70 min (SV3T3) or 90 min (Py3T3), the cells were fixed in 1% glutaraldehyde in phosphate buffered saline, and the number of cells with particle-free areas around them were counted in a Leitz Diavert inverted microscope with darkfield illumination.

Assay for the probing function of filopodia

The assay has been described elsewhere (Albrecht-Buehler 1976a).

Phagokinetic track assay

This assay has been described in detail elsewhere (Albrecht-Buehler 1977b).

Results

Movement of surface projections of freshly plated cells

As demonstrated earlier (Albrecht-Buehler and Lancaster, 1976), it is possible to quantitate the motile activity of surface projections in freshly plated cells on a substrate that is coated with colloidal gold particles. The projections adhere to the gold particles and transport them centripetally to the cell body, irrespective of the type of surface projection. Consequently, freshly plated cells with functional surface projections removed the gold particles within their reach and produced ring-shaped particle-free areas around the area on the substrate upon which they settled. Quantitation of the motile activity of surface projections was accomplished by counting the number of cells that were able to produce the particle-free rings in a certain time. We used this assay to quantitate the activity of surface projections of Py3T3 and SV3T3 cells, and compared it to the activity of the parental 3T3 cells, which had been published earlier (Albrecht-Buehler and Lancaster, 1976).

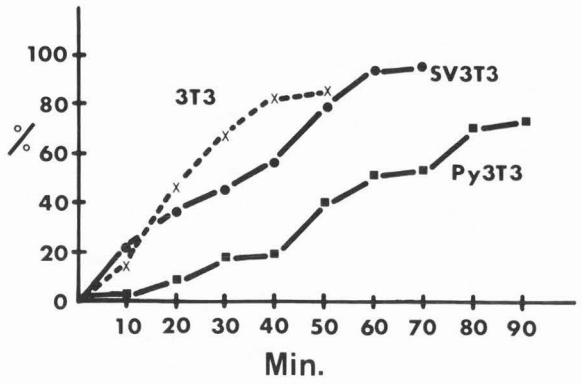

Figure 1: Percentage of freshly plated 3T3, Py3T3 and SV3T3 cells with particle-free rings around them as function of time. Culture medium: DME + 10% calf serum (CS).

Figure 1 shows the percentage of cells with particle-free rings around them as a function of time after settling on gold particle coated glass cover-slips in normal culture medium. The curves appear to rise exponentially to a plateau. The plateaus of the curves rose slightly, and eventually 100% of the cells were able to remove the gold particles around them. Transformed cell lines removed particles about half as fast as parental 3T3 cells. The speed of removal was 1 +/- 0.2 %/min for 3T3 cells, 0.5 +/- 0.2 %/min for Py3T3 cells and 0.7 +/- 0.2 %/min for SV3T3 cells at half maximal levels. However, SV3T3 cells reach a higher plateau value (95%) of the removal curves than 3T3 cells (85%) and Py3T3 cells (75%).

Figures 2a and 2d show the

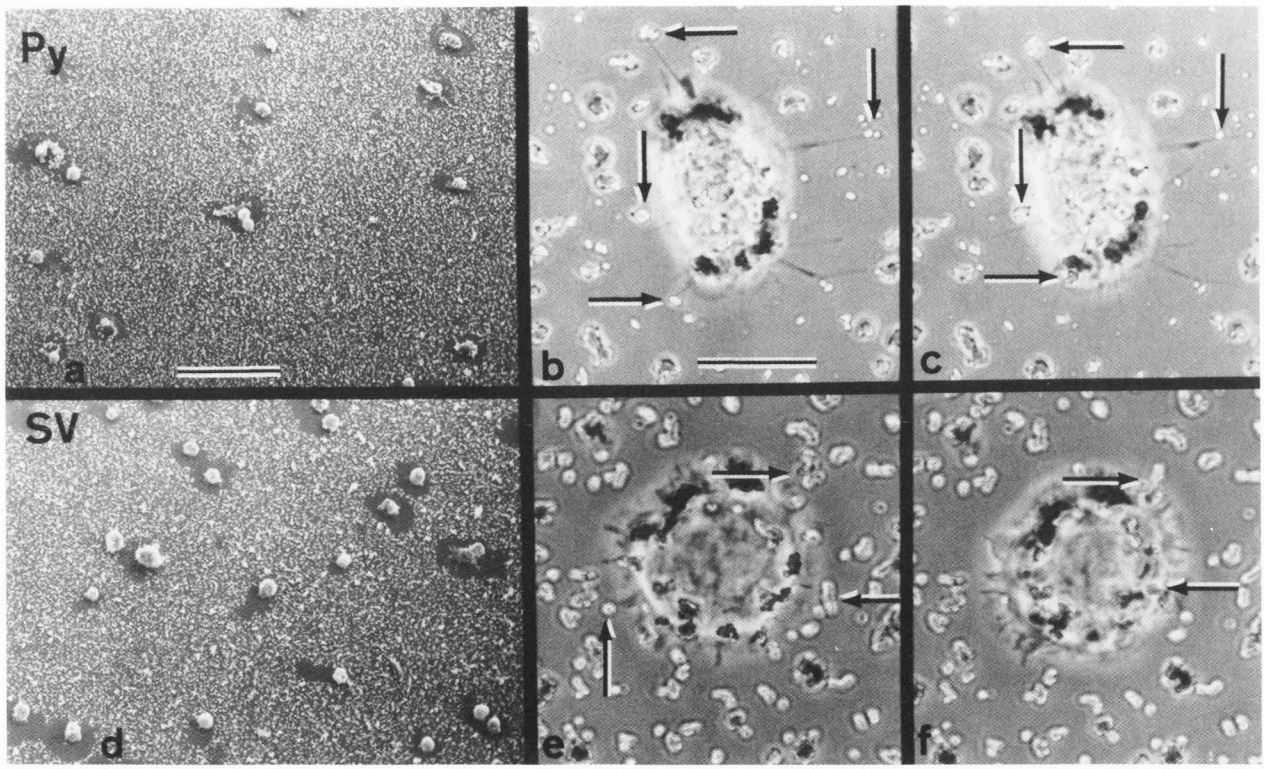

Figure 2: Particle removal by Py3T3 and SV3T3 cells.

a: Scanning electron micrograph of a field of Py3T3 cells 80 min after plating in DME + 10% CS. Tilt angle: 45°.Bar = 100 µm.

b,c: Removal of gold particles by filopodia of Py3T3 cells in DME + 10% CS. Times after plating 25 min and 26 min 10 s. Bar = 20 µm. Note the centripetal particle movement along a stretched out filopodium without its retraction (right hand arrow)

d: Scanning electron micrograph of a field of SV3T3 cells 70 min after plating in DME + 10% CS. Tilt angle: 45°. Magnification as in 2a.

e,f: Removal of gold particles by surface projections of SV3T3 cells in DME + 10% CS. Times after plating 25 min and 28 min 30 s. Magnif. as in 2b, 2c.

particle-free rings that were produced by both transformed lines in the presence of DME + 10% CS. Both 3T3 cells and Py3T3 cells were able to remove the gold-particles with filopodia by one of two mechanisms. Particles, either moved centripetally along a stretched out filopodium (right arrow in Figs. 2b,c), or the entire filopodium retracted together with an attached particle towards the cell body (lower left arrow in Figs. 2b,c). Both types of particle removal could be observed in the same cell (Figs. 2b,c). In contrast, SV3T3 cells removed the particles predominantly by extension and retraction of blebs, although several SV3T3 cells were observed which produced short, pointed processes that seemed intermediary between filopodia and lamellipodia (Figs. 2e,f). The speed of particle movement was estimated to be slower in the transformed (typical 0.1 μm/s) than in the parental 3T3 cells (typical 0.2 μm/s (Albrecht-Buehler and Goldman, 1976). However, it is difficult to measure these speeds accurately, because the particles move no more than a few micrometers and the movement has a 3-dimensional component, which is not viewed in the 2-dimensional microscopic images.

If the culture medium was replaced by normal salt solution, cells from both lines produced large spiky processes which removed the particles similar to normal 3T3 cells (cf. Figs. 6b,d)

Sensitivity of particle removal to extracellular conditions

The effects of various extracellular conditions were tested at the time when the curves describing the particle removal reached their plateau (cf. Fig. 1). Correspondingly, the sampling time after plating of the cells was 70 min in the case of SV3T3 cells and 90 min in the case of Py3T3 cells. In all cases the cells were washed twice in washing solution and plated into the experimental salt solution.

The presence of Mg^{++} or Ca^{++} in concentrations up to 1 mM had no effect on particle removal, whereas normal 3T3 cells were stimulated by both of the divalent cations. If potassium was omitted, particle removal by SV3T3 cells was unaffected, whereas particle removal by Py3T3 cells dropped to half maximal level, which was similar to normal 3T3 cells. Likewise, SV3T3 cells did not require Na-phosphate for maximal particle removal, whereas Py3T3 cells required 7 mM Na-phosphate (pH 7.4) and 3T3 cells 1 mM Na-phosphate (pH 7.8). In fact, SV3T3 cells were able to remove particles at maximal levels even in isotonic NaCl-solution.

The temperature dependence of particle removal in normal salt solution is shown in Fig.3. Similar to the results

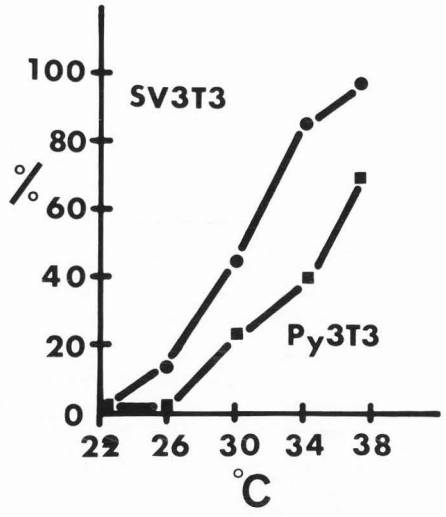

Figure 3: Temperature dependence of the percentage of cells with particle-free rings in normal salt solution. Cell were washed 2x in washing solution before plating. The corresponding data for 3T3 cells are published in Albrecht-Buehler and Lancaster, (1976).

with normal 3T3 cells, particle removal was inhibited below 22°C. However, surface projections were still formed by the cells at this temperature as shown in Fig. 4a,b.

Particle removal was also inhibited by the presence of 2.5 μg/ml cytochalasin B in normal salt solution, although the cells were able to extend surface projections (Fig. 4c,d). Similar results were obtained with normal 3T3 cells. The surfaces of 3T3 cells, Py3T3 cells and SV3T3 cells were remarkably smooth in normal salt solution containing 2.5 μg/ml cytochalasin B (Fig. 4c). However, the altered surface morphology was not the cause of the inhibition of particle removal because addition of 10 % calf serum restored the normal surface morphology, but it did not restore the ability of the cells to remove particles (Fig. 4d).

Particle removal by Py3T3 cells was partially inhibited if more than 5 μg/ml L-cystine was added to the normal salt solution. Similarly, 1 mg/ml of bovine serum albumin inhibited particle removal in Py3T3 cells. The results were quantitatively identical to those of 3T3 cells (Albrecht-Buehler and Lancaster 1976). In contrast, SV3T3 cells were unaffected by either addition.

SV3T3 cells also differed from 3T3 cells and Py3T3 cells in their sensitivity to Na-azide (Fig.5). Particle removal by 3T3 cells and Py3T3 cells was 50% inhibited by 0.5 mM Na-azide (Fig. 6a), whereas SV3T3 cells required 3.5 mM for the same level of

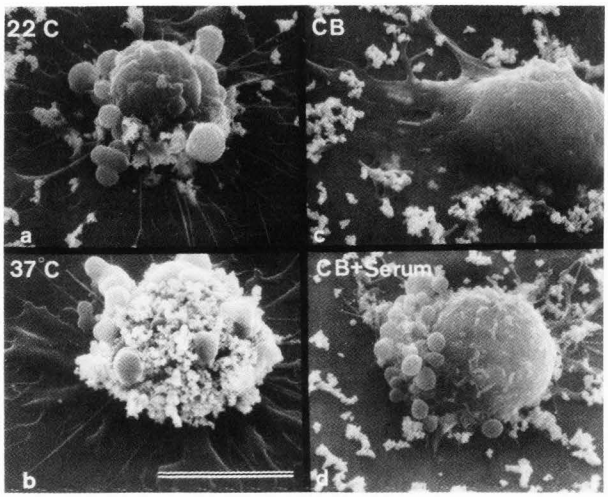

Figure 4: Morphology of SV3T3 cells on test substrates 70 min after plating in normal salt solution. Bar = 10 µm.
a: T = 22°C. The cell extends surface projections without removing particles.
b: T = 37°C. Similar surface morphology with accumulation of removed particles on the surface.
c: Smooth cell surface and surface projections after 60 min incubation in 2.5 µg/ml cytochalasin B.
d: Normal surface morphology without particle removal in the presence of 2.5 µg/ml cytochalasin B and 10% CS.

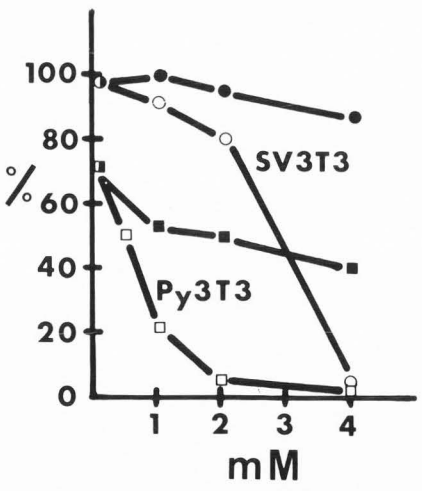

Figure 5: Inhibitory effect of Na-azide on particle removal during 70 min incubation of Py3T3 and SV3T3 cells (open symbols). Restoration of particle removal by the addition of 4.5 mg/ml glucose (closed symbols)

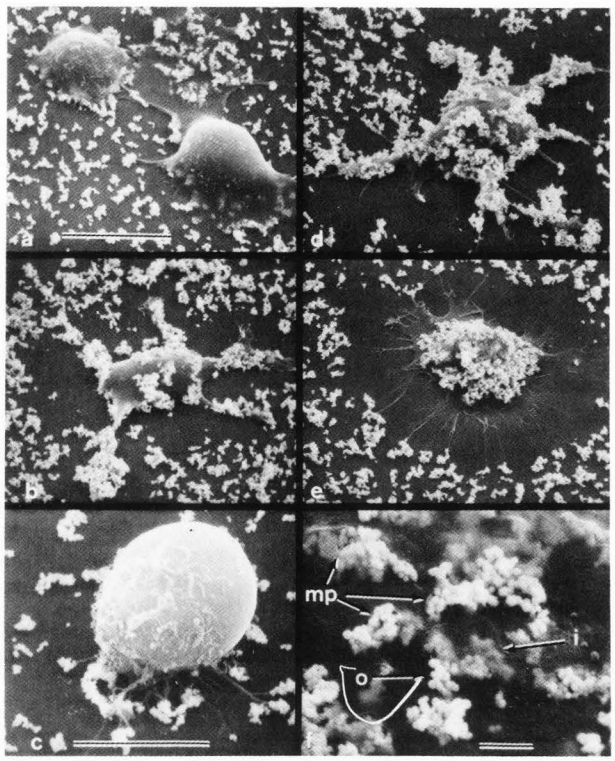

Figure 6: Surface morphology of Py3T3 and SV3T3 cells in the presence of Na-azide in normal salt solution 70 min after plating. Cells were washed twice in washing solution. Bars = 20 µm (a,b,d,e), 10 µm (c) and 2 µm (f). Tilt angle: 45°
a: Inhibition of particle removal by Py3T3 cells in 2 mM Na-azide
b: Particle removal by a Py3T3 cell in the presence of 4 mM Na-azide and 4.5 mg/ml glucose.
c: Partially smooth cell surface of a SV3T3 cells in the presence of 4 mM Na-azide
d: Particle removal by SV3T3 cells in the presence of 2 mM Na-azide.
e: Particle removal by SV3T3 cells in the presence of 4 mM Na-azide and 4.5 mg/ml glucose.
f: Phagocytosis of particle clusters on the surface of SV3T3 cells under the conditions of e). i: ingested particles; o: particles still on the outside of the cell; mp: membrane pockets closing around the particle clusters.

inhibition (Fig. 6d). In all cases the addition of 4.5 mg/ml glucose to 4 mM Na-azide in normal salt solution restored normal particle removal (Fig. 5, 6b, 6c), including phagocytosis of the removed particles (Fig. 6f). Na-azide altered the surface morphology of the cells. At concentrations of 4 mM Na-azide in normal salt solution the cell surfaces in all three cell lines were entirely smooth or contained large smooth patches (Fig. 6).

The pH dependence of particle

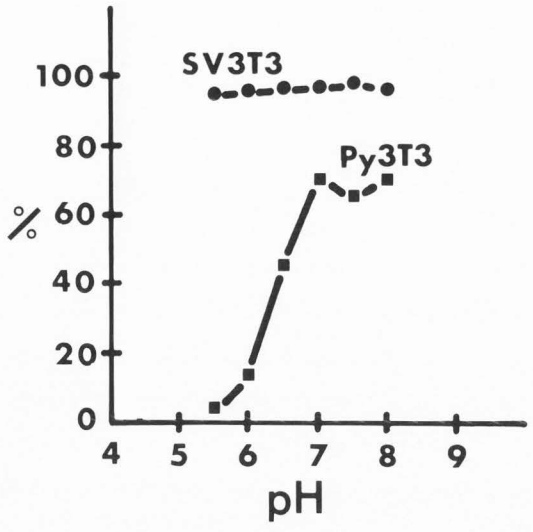

Figure 7: Dependence of particle removal on pH during 70 and 80 min after plating in normal salt solution. Cells were washed twice in washing solution.

removal in normal salt solution is shown in Figure 7. The response of Py3T3 cells to changes of pH was similar to that of normal 3T3 cells. In contrast, particle removal by SV3T3 cells showed a striking insensitivity to pH in the range of 5.5 to 8.5.

Recognition of an adjacent alternative substrate

We have reported earlier that contact of filopodia of 3T3 cells on glass with an adjacent area of evaporated gold on glass can induce the preferential spreading of a freshly plated 3T3 cell towards that alternative substrate (Albrecht-Buehler, 1976a). The results suggested a probing function of filopodia for suitable anchorage points around the cell. Using the same assay, we tested both transformed cell lines for the presence of a probing function of their filopodia or of other surface projections.

Although Py3T3 cells were able to produce filopodia which were immobilized upon contact with the gold surface (Fig. 8a), only 1 in 12 cells reacted to the contact by polarized spreading towards the gold substrate. The other cells spread without showing any preference for the alternative substrate (Fig. 8b-d)

In contrast, all 10 individually observed SV3T3 cells moved rapidly into the gold-plated areas following a contact of their surface projections with the gold surface (Fig. 8e-g). Their movement towards the gold was unusual for cultured cells because the shape of the locomoting cells was still quite spherical, which suggested that cell locomotion in culture does not require prior spreading. One hour after plating, most SV3T3 cells had moved away from the glass onto the gold-plated areas while the cellular outlines followed the borders of the areas (Fig. 8h).

Phagokinetic tracks of the transformed cells

We have published earlier, that the phagokinetic tracks of normal 3T3 cells expressed directional migration and mirror-symmetry or identity between the tracks of sister cells (Albrecht-Buehler, 1977a). In contrast, the tracks of SV3T3 cells and Py3T3 cells did not show any of these characteristics. Both cell lines produced rather erratic tracks. Relationships between sister cell tracks could not be detected. In general, the SV3T3 cells produced shorter tracks than did the Py3T3 cells during the same 1 day interval, which suggests that they moved more randomly, and/or more slowly than the parental 3T3 cells. In addition, the tracks of SV3T3 cells were rather featureless, whereas the Py3T3 cells produced many side extensions that removed gold particles at the sides, which gave their tracks a thorny appearance (Fig. 9).

The results of the entire comparison between the three cell lines are summarized in Table 1 in the form of an overview.

Discussion

The three major expressions of malignant tumors, cell growth, vascularization and tissue invasion, are also vital expressions of normal tissues. In the adult organism, rapid cell growth occurs normally in bone marrow, intestinal epithelium, epidermis and other epithelia. Vascularization plays an important role in the normal defense mechanisms during inflammation and wound healing. Invasive cell migration occurs regularly by various types of leukocytes. Therefore, during malignant invasion and tumor growth, nothing may be wrong with these cellular expressions per se, except that the cell type which expresses them, the time of expression, and/or the location of the expression are damaging for the organism. In short, the major defect in the expression of malignancy may not be the cellular actions, but the context in which they occur.

There seem to be two major theoretical possibilities to explain how cells can carry out a normal function in an abnormal context. They either sense their environment incorrectly, and thus take inappropriate actions to the stimulus, or their behavioral programs are altered, which leads to an abnormal response to the otherwise correctly sensed environment. For example, it seems conceivable, and has been suggested in the past, that malignant cells lose

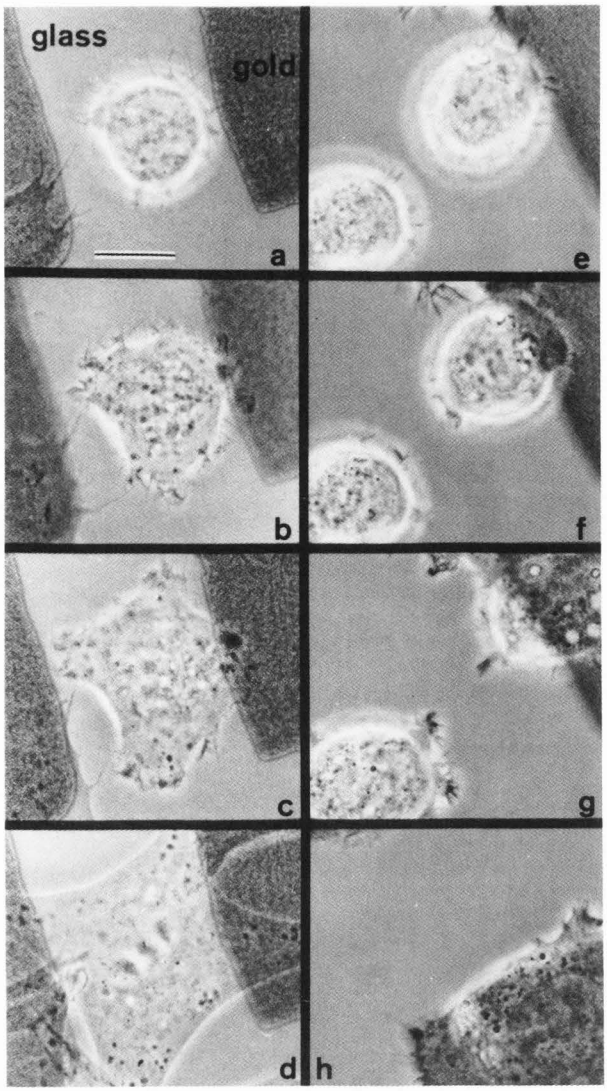

Figure 8: Recognition of an adjacent gold-plated area on the substrate after contact by surface projections of SV3T3 cells (e-h) in DME + 10% CS, and the absence of a similar behavior in Py3T3 cells (a-d). Bar = 20 μm. Observation times are for Py3T3 cells, a: 30.5 min, b:36 min, c: 43 min, d: 101 min, and for SV3T3 cells, e: 58.5 min, f:60 min, g: 67 min, h: 92 min. Note the locomotion of the cell before it spreads on the substrate.

certain surface receptors for growth factors. Alternatively, the receptors may be present, but their interaction with the ligands may lead to an abnormal reaction of the cell. The two possibilities do not necessarily apply only to the invading tumor cell, but the invaded tissue may also be affected in similar ways. In several cases of in vitro invasion, it seems that the invaded tissue cooperates with the invading cell.

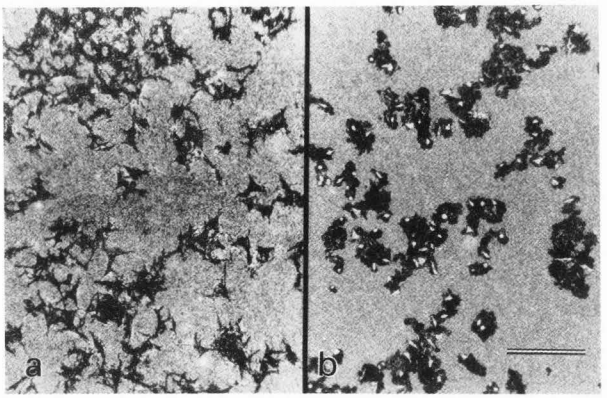

Figure 9: Phagokinetic tracks of a: Py3T3 cells and b: SV3T3 cells 24 hours after plating in DME + 10% CS. Bar = 100 μm.

For example, invasion of the chick chorioallantoic membrane (CAM) by malignant test cells (Hart and Fidler, 1978) should lead to the disintegration of the CAM if the invading cells would perforate it. Instead, the CAM remains intact throughout the experiment, suggesting that the epithelial cells of the CAM seal behind the invading cells (cf. Nicholson, 1982). The penetration of the endothelium by individual tumor cells leaves the vessel intact (De Bruyn, 1983), suggesting that the invaded cells continue to resist successfully the capillary pressure.

The above discussion treats tissue cells as if they were 'intelligent' systems, capable of global assessment of their environment, of making decisions and of coordinating complex functions of their bodies for a purpose such as invasion (Albrecht-Buehler, 1985). This notion is in contrast to the generally accepted concept that tissue cells are not required to make choices, but are under the automatic and irrefutable dictate of hormones, growth factors, environmental concentrations of metabolites, numbers of adhesion and recognition sites, and the biochemical pathways that are activated following the occupation of receptors with the proper ligand. If, on the other hand, the concept of 'intelligent cells' is correct, and tissue cells, indeed, make decisions that can affect the well-being of the entire organism, then it would be crucial, that they are able to receive the relevant signals from their environment and apply a certain inherited 'normal logic' as they interpret the signals and react to them.

The observations presented in this paper seem to offer several examples of altered sensitivity of transformed cells. For example, the continuation of particle

Table 1

Summary of the comparison between the motile behavior of 3T3, Py3T3 and SV3T3 cells.

Parameter	3T3	Py3T3	SV3T3
removal at 50%(%/min)	1	0.50.	7
plateau (%)	85	75	95
temperature ($22^{o}C$-$37^{o}C$)	sensitive	sensitive	sensitive
cytochalasin B (0-5 µg/ml)	sensitive	sensitive	sensitive
Na-azide (0-4 mM)	sensitive	sensitive	less sensitive
bovine serum albumin (0-1 mg/ml)	sensitive	sensitive	insensitive
Na-phosphate (0-30 mM)	sensitive	sensitive	insensitive
pH (5.5-8.5)	sensitive	sensitive	insensitive
L-cystine (0-10 µg/ml)	sensitive	sensitive	insensitive
discrim. betw. Na and K	yes	yes	no
Ca^{++}, Mg^{++}	sensitive	insensitive	insensitive
recognition of adjacent substrate	yes	no	yes
phagokinetic tracks	persistent	irregular	irregular
symmetry betw. sister cells	yes	no	no

removal by SV3T3 cells at pH= 5.5 is in pronounced contrast to the complete inhibition of particle removal of 3T3, and Py3T3 cells at this pH value. The SV3T3 cells seem to behave in an acidic medium similar to macrophages which spread better in acidic than in alkaline environments (Rabinovitch and DeStefano, 1973). The altered sensitivity to pH of transformed cells also seems to be expressed in their growth characteristics (Ceccarini and Eagle, 1971). Similarly, the insensitivity of particle removal by Py3T3 and SV3T3 cells to extracellular calcium and magnesium may be another example of altered sensitivity of cells following transformation. As in the case of altered sensitivity to pH, altered calcium requirements for the growth of transformed cells have also been reported (Boynton and Whitfield, 1976).

Although certain transformed cells may have altered sensitivities to environmental factors such as pH and extracellular calcium concentration, it is also possible, that their sensitivity to other factors is normal, but that their responses to them are altered. Py3T3 cells seem to present an example for altered behavior. Judging by the removal of gold particles upon contact by a filopodium or another surface projection, the Py3T3 cells are able to sense the contact with a gold particle and induce a retraction of the surface of the projection or of the entire projection that transports the particle to the cell body. In this respect they behave identical to normal 3T3 cells (Albrecht-Buehler and Lancaster, 1976) and SV3T3 cells. Consequently, one may assume, that the same retraction occurs, if the filopodium of a Py3T3 cell touches an adjacent area of evaporated gold, except that the attached filopodium can only retract isometrically. We have argued earlier (Albrecht-Buehler 1976b) that the isometric retraction of a filopodium may be the signal for the cell to spread towards the attached tip of the filopodium. Thus, cells with this behavioral rule will spread preferentially towards the area of evaporated gold. However, in contrast to 3T3 cells and SV3T3 cells, the Py3T3 cells do not translate the contact signal into a preferential movement into the gold-plated area. It seems possible that the rules of their behavior have been changed.

If one had to decide which of the two transformed 3T3 cell lines is 'more' transformed, one would probably choose the SV3T3 over the Py3T3 cell line based on their larger number of insensitivities to environmental factors (Table 1). Yet, compared to the parental 3T3 cells and the 'less' transformed Py3T3 cells, they are less motile or less persistent in their locomotion. They were only faster in the case of stimulated migration towards a gold plated area which they had touched with a surface projection. This observation also emphasizes the caution expressed in the introduction to this paper, namely that malignant cells may not be more motile, but rather they are altered in their responses to environmental clues.

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Discussion with reviewers

B. R. Brinkley: The ability of filopodia to probe the tissue culture environment for points of anchorage appears to be a logical assessment of their contact and invasiveness. I would like to know more explicitly why the author chose this particular model. What feature of Py3T3 would make it respond differently from SV3T3 or the 'normal' counterpart 3T3? Why not use a Ts transformed line where normal and transformed phenotypes can be monitored at the permissive and restrictive temperatures?

Author: I have chosen the 3T3 system, because a great deal is known about their motility, cytoskeleton and biochemistry. A temperature-sensitive mutant was not included in the study, because cell motility is directly temperature-dependent. Consequently, a change of temperature can be expected to alter motility together with the transformed phenotype.

B. R. Brinkley: Aside from the fact that SV3T3 reaches a higher plateau value, there does not appear to be any significant differences between 3T3 and SV3T3 in the capacity to move gold particles from the substrate (Fig. 1). Are the actual values statistically different?

Author: Each point on the curves is based on counts of 250 cells in two different experiments. Individual counts differed 11% on average between the experiments. Therefore, individual points of curves showing particle removal by 3T3 and SV3T3 cells are statistically different only at 30 and 40 min. In other words, at 35 min most 3T3 cells have removed the particles around them, whereas hardly half of the SV3T3 cells have produced the same result. This is quite obvious in the preparations.

R. M. Albrecht: The author suggests abnormal sensing or an abnormal response to normal sensing as possible causes of the observed changes in the response. Also mentioned are co-operative responses of tissues to the abnormal or altered cells, as though the "normal" tissue were trying to accommodate or work with the "abnormal" cells. (The aiding and abetting syndrome) Could the author consider the changes, in the "transformed" cells, in the context of normal developmental or normal regenerative processes? That is, the "altered" cell may be both correctly sensing and correctly responding but is living in the wrong place, ie. what are the possibilities of there being any "right places" for these cells? If plugged into the correct place and time in developing or regenerating systems these cells could possibly have "normal" responses. Probably not, but does the author have any comments on this observation?

Author: I agree with the reviewer that cells in tissue culture are probably permanently in the "wrong places". It is quite possible that the appropriate embryonic environment may make "wrong" reactions of transformed cells "right". The possibility to produce normal mice from blastocysts which contain teratocarcinoma cells (Mintz and Illmensee, 1975) seems to support the possibility that transformed cells can behave normally in the presence of normal cells. The above data can merely show that different transformants responded differently to the identical physical reality in their tissue culture environment as if they sensed it differently, or interpreted it differently.

(Reference: Mintz B, Illmensee K (1975). Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc. Natl. Acad. Sci. USA 72: 3585-3589)

M. Rabinovitch: I understand the point, since computers can also be intelligent, but I dislike ascribing "intelligence" to cells. Laypersons are confused enough with "intelligent" electrons, plants that grow with music, rocks that are alive...Do we need to have your journal quoted as providing evidence that cells think? Sure""""""" help, but "intelligent" means so many different things to different people. And then somebody will soon claim that SV 3T3 are more intelligent than mouse macrophages or even Leishmania parasites. That would be hard to bear.

Author: I have defined the term of 'intelligence' in the context of cells (Albrecht-Buehler, 1985), and cannot be concerned with its potential abuse. Many other common words have survived their use in science without degrading it, provided they described a reality, e.g. 'information', 'fields', 'strangeness' and 'messenger'. If 'cell intelligence' is a reality, i.e. if cells are able to process data, make decisions and integrate multiple cytoplasmic functions for a biological purpose, then I see no reason to avoid the word. For the past twelve years I have worked in cell behavior with the sole purpose to test this notion of cell intelligence. I submit to the reviewer that many of the experiments which I published over the years support the possibility that it describes a reality.